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STAT3 Mediates Autophagy and Extracellular Matrix Synthesis in Human Glomerular Mesangial Cells Exposed to High Glucose

Y. S. Yang, W. L. N. Wang, C. Y. Chen, G. X. R. Gao, S. D. Sun
The First Hospital of China Medical University, Shenyang, Liaoning, China

Objectives: To explore the effect of STAT3 pathway on the autophagy of human glomerular mesangial cells (HGMC) and expression of extracellular matrix Collagen IV cultured in high glucose.

Methods: HGMC were cultured in normal (5 mmol/L) or high glucose (30 mmol/L) for 12–72 hours, and then S31-201 (an inhibitor of STAT3 activity) or STAT3-siRNA transfection were added into HGMC. STAT3, p-STAT3 and autophagy marker proteins LC3 and p62 protein expression was evaluated by western blot, autophagosomes were detected by electron microscopic and the expression of collagen IV was detected by ELISA in each group.

Results: High glucose significantly reduced the generation of autophagosome and altered expression of LC3 and p62 at 24 h (LC3 II/LC3I and LC3II decreased, p62 increased obviously), consistently high glucose exposure significantly increased protein levels of STAT3, p-STAT3 and collagen IV. These changes were reversed by S31-201 or STAT3-siRNA transfection.

Conclusion: High glucose can inhibit autophagy activity of human glomerular mesangial cells and induce up-regulation expression of Collagen-IV. Inhibition of STAT3 pathway can reduce the inhibition of autophagy activity and synthesis of collagen IV by high glucose.

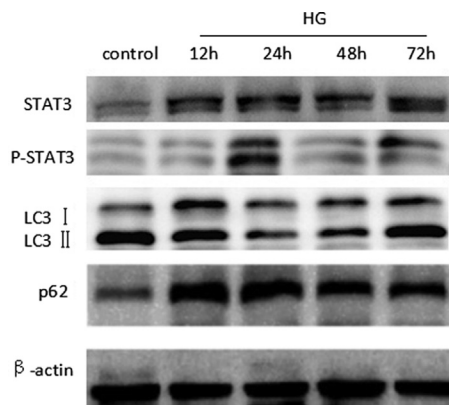


Fig. 1 High glucose inhibits autophagy activity and induces STAT3 activation of human glomerular mesangial cells at 24 h.

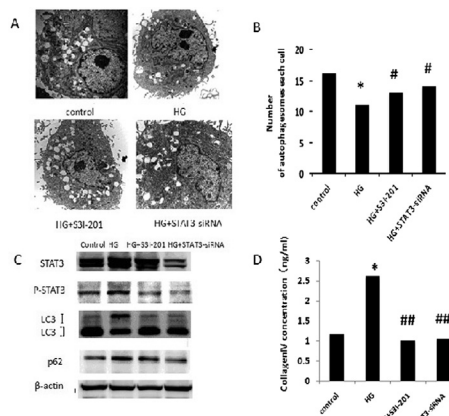


Fig. 2 Inhibition of STAT3 pathway reduces the inhibition of autophagy activity and synthesis of collagen IV by high glucose. (A, B) Autophagosomes were detected by electron microscopic (* $P < 0.01$ vs control, # $P < 0.05$ vs high glucose). (C) Analysis of stat3, p-stat3, LC3 and p62 by Western blot. (D) Expression of Collagen IV was detected by ELISA (* $P < 0.01$ vs control, ## $P < 0.01$ vs high glucose).

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0212

Ursolic Acid Improves the Podocytes Injury Caused by High Glucose

Li Xu, Qiuling Fan
The First Hospital of China Medical University, Shenyang, Liaoning, China

Objective: Autophagy plays an important role in maintaining podocytes homeostasis. Reduced autophagy may result in limited renal cell function during exposure to high glucose. In this study, we investigated the effects of ursolic acid (UA) on autophagy and podocytes injury induced by high glucose.

Methods: Conditionally immortalized murine podocytes were cultured in media containing high glucose, and we determined the effect of the PI3K inhibitor LY294002 and ursolic acid treatment on protein expression. miR-21 expression was detected using RT-qPCR. Activation of the PTEN-PI3K/Akt/mTOR pathway, expression of autophagy-associated proteins and expression of podocytes specific proteins were determined by Western blot. Immunofluorescence was used to monitor expression of podocytes specific proteins and LC3 accumulation. Autophagosomes were observed using electron microscopy.

Results: During exposure to high glucose condition, autophagy was reduced in podocytes. Increased miR-21 expression, decreased PTEN expression, and activation of the PI3K/Akt/mTOR pathway were observed during exposure to high glucose. Ursolic acid and LY294002 reduced podocytes injury through autophagy rescue. Our data suggest ursolic acid inhibits miR-21 expression and increases PTEN expression, thereby inhibiting Akt and mTOR and restoring autophagy levels.

Conclusion: Our data suggest that podocytes injury is associated with reduced autophagy levels during exposure to high glucose. Ursolic acid reduces podocytes injury by increasing autophagy through miR-21 inhibition and PTEN expression.

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0227

Influence of Shenkang Injection on Hemodynamics of Kidney in Patients with Early Diabetic Nephropathy

Rao Fu
The first hospital of Harbin City, Harbin, China

Objective: To observe the influence of Shenkang injection on renal artery peak velocity in systole (Vs) and resistance indices (RI) of patients with early diabetic nephropathy.

Methods: A total of 58 early diabetic nephropathy patients were randomly divided into conventional treatment group (A group) and Shenkang Injection-treated group (B group), 29 cases in each group. To detect the level of UAER between before and after treatment, color Doppler ultrasound were used to check and analyze the chief renal arteries and intersegmental arteries Vs and RI of patients. Moreover, 25 healthy subjects were measured for control.

Results: (1) Vs of patients with early diabetic nephropathy was significantly lower than that in normal control group, RI was significantly higher than that in normal control group ($P < 0.01$). (2) Vs of B group was significantly higher than that before ($P < 0.01$), and RI was significantly lower than that before ($P < 0.05$). All the improvements were better than those in A group. The A group had no significant difference with treated before ($P > 0.05$). UAER of B group was significantly lower than that before and better than that of A group ($p < 0.01$).

Conclusion: Shenkang injection can increase renal artery peak velocity in systole, decrease resistance indices, improve renal ischemia state, decrease urine protein, and has curative effect on diabetic nephropathy in earlier stage.

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0264

Effect Of Glucagon-like Peptide-1 Receptor Agonists on Regulation of Blood Pressure and Water-Sodium Metabolism in Rats with Diabetic Nephropathy

Shaoqing Wang
Chengdu Medical College, Chengdu, China

Objective: To observe the effect of glucagon like peptide-1 receptor agonist liraglutide on blood pressure and water-sodium metabolism in rats

with diabetic nephropathy and investigate the relationship between glucagon like peptide-1 receptor agonist and the expression of COX2 in renal medulla.

Methods: 130 male Sprague Dawley rats were divided into the conventional feed group (normal group, $n = 30$) and high sugar and fat diet fed group ($n = 100$) randomly. Diabetic rats were induced by intraperitoneal injection of streptozotocin after high sugar and fat diets fed eight weeks. The diabetic rats ($n = 72$) divided into the diabetic nephropathy (DN) group, Low-Liraglutide (L-Lg, 5 $\mu\text{g/kg}$) group and High-Liraglutide (H-Lg, 10 $\mu\text{g/kg}$) group. To measure the blood pressure, the level of sodium, 24-hour urinary sodium (UNa) and 24-hour urinary 6-keto-prostaglandin $\text{F}_{1\alpha}$ (6k-PGF $_{1\alpha}$). Determination of the expression of COX2 in renal medullary tissue using immunohistochemistry. Using Western blot technology analysis the expression of COX2 protein in the renal medullary.

Results: Compared with N Group, the blood pressure, the serum sodium and UNa are increasing, the urinary 6k-PGF $_{1\alpha}$ is decreasing, the renal medulla expression of COX2 is increasing in DN group ($P < 0.05$). Compared with the DN group, the blood pressure, the serum sodium and UNa are decreasing, the urinary 6k-PGF $_{1\alpha}$ is increasing, the renal medulla expression of COX2 is still increasing in L-Lg group and H-Lg group ($P < 0.05$). Compared with L-Lg group, the renal medulla expression of COX2 is further increasing ($P < 0.05$). **Conclusion:** GLP-1 receptor agonist Liraglutide can reduce the blood pressure, the serum sodium, increase the excretion of 24-hour urinary sodium excretion in DN rats, and increase the excretion of 24-hour urinary 6k-PGF $_{1\alpha}$, and upregulate the expression of COX2 protein in the renal medulla. The mechanism of GLP-1 receptor agonist regulate blood pressure and water-sodium metabolism by activation of COX2.

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0268

Different Doses of Heparin in the Application of Continuous Renal Replacement Therapy for Diabetic Nephropathy

W. Y. Wang, W. J. Q. Wang, W. W. G. Wang, L. W. Liu, Z. W. J. Zhang
Lanzhou University Second Hospital, Lanzhou, China

Objective: Compare the efficacy and safety of anticoagulation with different doses of heparin in the continuous renal replacement therapy (CRRT) for diabetic nephropathy, confirm the effective dose of anticoagulant in the treatment of CRRT for diabetic nephropathy to provide the basic data for clinical work.

Methods: 54 patients with diabetic nephropathy were randomly divided into three groups; group 1 (18 patients, heparin, 0.3 mg/kg), group 2 (18 patients, heparin, 0.4 mg/kg), group 3 (18 patients, heparin, 0.5 mg/kg). To evaluate coagulation function, the filter and pipeline were observed; the activated partial thromboplastin time (APTT) and platelet levels were monitored before or after treatment.

Results: In group 1, the APTT and platelet levels had no significant change after treatment; in group 2, the APTT extended to 1.5 times with no significant change of platelet levels; in group 3, the APTT raised obviously, otherwise, the platelet levels decreased remarkable with significance among group 1, group 2 and group 3 ($P < 0.05$).

Conclusion: As for diabetic nephropathy, a larger dose of heparin could increase efficacy and safety of CRRT.

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0269

Downregulation of the Expression of PINCH-1 in Kidney Tissue by Irbesartan in Diabetes Mellitus Rat

Heping Zhang, Xiaohui Liu, Jiangchao Feng, Jie Zhang, Ling Lin
Affiliated Hospital of North Sichuan Medical College, Nanchong, China

Objective: To investigate the effect of irbesartan in regulating the expression of PINCH-1 in the kidney tissue of diabetes mellitus rat using diabetes mellitus rat model.

Methods: After establishing rat model of diabetes mellitus, real-time polymerase chain reaction (RT-PCR) and Western blot were applied to detect the expression of PINCH-1 in the kidney tissue of diabetes mellitus rat after irbesartan treatment.

Results: RT-PCR shown the PINCH-1 mRNA expression in diabetes mellitus rat (1.537 ± 0.04) was higher than in the normal rat (1.128 ± 0.03), and decreased after the treatment of irbesartan (1.246 ± 0.02) ($P < 0.05$). Western blot showed the expression of PINCH-1 in the kidney tissue of diabetes mellitus rat with irbesartan treatment (1.159 ± 0.03) ($P > 0.05$).

Conclusion: Irbesartan could downregulate the expression of PINCH-1 in the kidney tissue of diabetes mellitus rat.

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0273

Clinical Analysis of Type 2 Diabetes with Proteinuria

X. Z. Zheng

The 211 Hospital of PLA, Harbin, Heilongjiang Province, China

Objective: To analyze the kidney pathogenesis of proteinuria in patients with type 2 diabetes mellitus.

Methods: Selected 50 cases of type 2 diabetic patients with proteinuria, all of which were given tests as follows, echocardiography, carotid ultrasonography, fundus examination, glomerular filtration rate, renal function, blood lipids, blood glucose, glycosylated hemoglobin, urine analysis, 24 hour urinary protein quantitative clinical physical examination and renal biopsy. According to the pathological results, patients were divided into diabetic nephropathy group and non-diabetic nephropathy group.

Results: 48.2% of 50 cases were diagnosed as diabetic nephropathy, while 51.8% were diabetic patients with other glomerular diseases, namely non-diabetic nephropathy, and in which the highest proportion was focal segmental glomerulosclerosis. Fasting blood glucose was high in diabetic nephropathy group ($P < 0.05$). Cardiac color Doppler ultrasound indicated that the ejection fraction in the diabetic nephropathy group was significantly lower than that in the non-diabetic nephropathy group ($P < 0.05$). Carotid artery ultrasonography examination showed that in diabetic nephropathy group The number of atherosclerotic plaque patients was significantly higher than that in non-diabetic nephropathy group, while the intima media thickness (IMT) of the carotid artery in diabetic nephropathy group was higher than that in non-diabetic nephropathy group ($P < 0.05$). It is worth noting that the non-diabetic nephropathy has little to do with diabetic retinopathy, which means diabetic retinopathy has a high sensitivity and specificity for the diagnosis of diabetic nephropathy ($P < 0.01$).

Conclusion: in the case of type 2 diabetes complicated with urinary protein, fasting blood glucose, heart shot ejection fraction, carotid atheromatous plaque and intima-media thickness, and fundus changes could be used as therference indexes for the diagnosis of diabetic nephropathy and non-diabetic nephropathy, while renal biopsy is an important diagnosis index of diabetic kidney disease.

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0281

Clinical and Pathological Predictors of Tubulointerstitial Injury in New Pathological Classification of Diabetic Nephropathy

Xuejing Zhu¹, Xiaofen Xiong¹, Shuguang Yuan¹, Li Xiao¹, Xiao Fu¹, Yuan Yang¹, Chengyuan Tang¹, Fuyou Liu¹, Lin Sun^{1,2}

¹Second Xiangya Hospital of Central South University, Changsha, China

²Department of Pathology & Medicine, Northwestern University, Chicago, Illinois, USA

Objectives: To evaluate the new pathological classification of diabetic nephropathy (DN) published by Research Committee of the Renal Pathology Society in 2010 and to investigate clinical and pathological predictors of tubulointerstitial injury in new pathological classification.

Methods: 43 cases with DN performed renal biopsy. Patients were divided into different groups according to glomerular classification or interstitial fibrosis and tubular atrophy (IFTA) score by the new pathological classification. We used χ^2 test or Fisher's exact test, Mann-Whitney U-test, Kruskal-Wallis H-test and Spearman's correlation to make comparisons and correlations between the clinical and pathological findings